

REVIEW ARTICLE

Phospholipase C ζ (PLC ζ) versus postacrosomal sheath WW domain-binding protein (PAWP): Which molecule will survive as a sperm factor?

Michiko Nakai¹ | Junya Ito^{2,3}  | Ayumi Suyama³ | Atsuko Kageyama³ | Yasuko Tobari⁴ | Naomi Kashiwazaki^{2,3}

¹Institute of Agrobiological Sciences, National Agriculture and Food Research Organization, Tsukuba, Japan

²Laboratory of Animal Reproduction, School of Veterinary Medicine, Azabu University, Sagami, Japan

³Graduate School of Veterinary Sciences, Azabu University, Sagami, Japan

⁴Laboratory of Animal Genetics and Breeding, School of Veterinary Medicine, Azabu University, Sagami, Japan

Correspondence

Junya Ito, Laboratory of Animal Reproduction, School of Veterinary Medicine, Azabu University, Sagami 252-5201, Japan.
Email: itoj@azabu-u.ac.jp

Funding information

Japan Society for the Promotion of Science, Grant/Award Number: 15H04584 and 17K08054; Ministry of Education, Culture, Sports, Science and Technology

Abstract

During mammalian fertilization, sperm is fused with the oocyte's membrane, triggering the resumption of meiosis from the metaphase II arrest, the extrusion of the second polar body, and the exocytosis of cortical granules; these events are collectively called 'oocyte activation.' In all species studied to date, the transient rise in the cytosolic level of calcium (in particular, the repeated calcium increases called 'calcium oscillations' in mammals) is required for these events. Researchers have focused on identifying the factor(s) that can induce calcium oscillations during fertilization. Sperm-specific phospholipase C, i.e., PLC zeta (PLC ζ), is a strong candidate of the factor(s), and several research groups using different species obtained evidence that PLC ζ is a sperm factor that can induce calcium oscillations during fertilization. However, postacrosomal sheath Tryptophan-Tryptophan (WW)-domain-binding protein (PAWP) was recently shown to have a pivotal role in inducing calcium oscillations in some species. In this review, we focus on PLC ζ and PAWP as sperm factors, and we discuss this controversy: Which of these two molecules survives as a sperm factor?

KEYWORDS

calcium, fertilization, oocyte, phospholipase C, sperm

1 | SPERM FACTOR THEORY

Ovulated mammalian oocytes remain arrested until fertilization at the metaphase II (Ducibella, Schultz, & Ozil, 2006; Jones, 2005). In mammals, there are transient and repetitive increases in the cytosolic level of free calcium (in a phenomenon that is also known as calcium oscillations); these oscillations are induced immediately after sperm-oocyte fusion (Kline & Kline, 1992; Miyazaki, Yuzaki, et al.,

1992; Miyazaki et al., 1993). These calcium oscillations trigger the events of oocyte activation including the resumption of second meiosis, maternal mRNA recruitment, pronucleus (PN) formation, and embryonic development (Xu, Kopf, & Schultz, 1994). The pattern of rise such as amplitude and number in the cytosolic level of calcium might affect gene expressions at later stages of preimplantation embryo development and even after postnatal development (Ozil, Banrezes, Toth, Pan, & Schultz, 2006).

Michiko Nakai and Junya Ito were equally contributed.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2020 The Authors. *Animal Science Journal* published by John Wiley & Sons Australia, Ltd on behalf of Japanese Society of Animal Science.

The mechanisms by which sperm induce calcium oscillations have been investigated in several species. In the hamster (Miyazaki, Shirakawa, et al., 1992) and mouse (Xu et al., 1994) as well as echinoderms (Carroll et al., 1997) and frogs (Nuccitelli, Yim, & Smart, 1993), the sperm-induced calcium increase is generated by a release of calcium from 1,4,5-inositol trisphosphate (IP_3)-sensitive intracellular calcium stores. Since IP_3 is generated by a phospholipase C (PLC)-mediated hydrolysis of phosphatidyl inositol 4,5-bisphosphate ($PI(4,5)P_2$), the activation of PLC and the production of IP_3 are required for the induction of calcium oscillations. The details of the mechanisms underlying the calcium release at fertilization in vertebrates have not yet been established – especially the interaction between the sperm and oocyte in this process.

Three major hypotheses have been proposed to date to explain how sperm induce calcium oscillations during mammalian fertilization (Ito, Parrington, & Fissore, 2011; Kurokawa, Sato, & Fissore, 2004; Runft, Jaffe, & Mehlmann, 2002; Swann, Saunders, Rogers, & Lai, 2006; Whitaker, 2006). The first hypothesis is known as the 'membrane receptor hypothesis' and contends that an oocyte surface receptor is engaged by a sperm ligand, triggering the production of a signaling pathway that triggers calcium release via the PLC of the oocyte (reviewed by Swann & Parrington, 1999) (Figure 1a). The 'conduit hypothesis' proposes that extracellular calcium flows into the oocyte via the sperm during gamete fusion (Figure 1b). Both the membrane receptor hypothesis and the conduit hypothesis rely on a sperm-oocyte interaction to induce calcium responses, and the implausibility of these hypotheses was later revealed by the success of intracytoplasmic sperm injection (ICSI), which can bypass the sperm-oocyte interaction in the plasma membrane and is capable of inducing fertilization-like calcium responses.

A third theory, the 'sperm factor hypothesis,' could be considered a very likely mechanism for the induction of calcium oscillations (Figure 1c). The sperm factor hypothesis suggests that immediately after sperm-oocyte fusion, the sperm release a soluble activating factor into the oocyte (Stricker, 1999; Swann, 1996). In support of this hypothesis, the injection of sperm cytosolic extracts into oocytes resulted in calcium oscillations similar to those observed during fertilization in many species, including sea urchin (Dale & Santella, 1985), an ascidian (McDougall, Levasseur, O'Sullivan, & Jones, 2000), chicken (Kim & Gye, 2003), quail (Mizushima et al., 2009), hamster (Swann, 1990), mouse (Parrington, Swann, Shevchenko, Sesay, & Lai, 1996), rat (Ito, Shikano, Oda, et al., 2008), cow (Wu, He, & Fissore, 1997), pig (Machaty, Bonk, Kuhholzer, & Prather, 2000), human (Wilding, Kyozyuka, Russo, Tosti, & Dale,

1997), horse (Hinrichs, Choi, Love, Chung, & Varner, 2006), and rabbit (Stice & Robl, 1990). The sperm factor hypothesis is now strongly supported; it appears that one or more sperm factors exist on sperm, and the factor(s) are a trigger to induce calcium oscillations and oocyte activation – at least in mammalian species.

2 | THE DISCOVERY OF $PLC\zeta$

The calcium release component(s) of sperm cytosolic extracts has been investigated. A 33-kDa protein isolated from a sperm extract fraction that localizes in the equator of sperm and has the ability to activate oocytes: oscillin (Parrington et al., 1996), but a microinjection of recombinant oscillin does not induce calcium oscillations (Wolosker et al., 1998). Moreover, when sperm extracts were fractionated, oscillin was not detected in the fraction with strong calcium-releasing activity (Wu et al., 1997). A truncated c-kit tyrosine kinase (tr-kit) was also considered as a candidate sperm factor (Sette et al., 1997), and although a microinjection of tr-kit mRNA into mouse oocytes caused complete oocyte activation, it is not yet clear whether or not tr-kit has calcium releasing activity (Sette et al., 1997).

Saunders et al. (2002) proposed that phospholipase $C\zeta$ ($PLC\zeta$) might be the sperm-borne oocyte-activating factor that can induce calcium oscillations. A microinjection of RNA encoding $PLC\zeta$ (Saunders et al., 2002) or recombinant $PLC\zeta$ protein (Kouchi et al., 2004) induced calcium oscillations. In addition, the calcium oscillation-inducing activity of sperm extracts was lost when the sperm extracts were pre-treated with an antibody against $PLC\zeta$ (Saunders et al., 2002). The level of $PLC\zeta$ that is able to produce fertilization-like calcium oscillations in a single oocyte is in the same range as the single-sperm content of $PLC\zeta$ (Saunders et al., 2002). Moreover, reducing the level of sperm $PLC\zeta$ protein by transgenic RNA interference resulted in the perturbation of the patterns of calcium oscillations after fertilization (Knott, Kurokawa, Fissore, Schultz, & Williams, 2005).

To date, $PLC\zeta$ has been detected in many mammalian and non-mammalian species (Table 1), including mouse (Ito, Shikano, Kuroda, et al., 2008; Saunders et al., 2002), rat (Ito, Shikano, Oda, et al., 2008), human (Cox et al., 2002; Ito, Shikano, Oda, et al., 2008), cynomolgus monkey (Cox et al., 2002), cow (Cooney et al., 2010; Malcuit et al., 2005), pig (Kurokawa et al., 2005; Yoneda et al., 2006), horse (Bedford-Guaus et al., 2011; Sato et al., 2013), medaka fish (Coward et al., 2011), and quail (Mizushima et al., 2014). $PLC\zeta$ orthologues were identified even in pufferfish species *Takifugu rubripes* (fugu) and *Tetraodon nigroviridis* (tetraodon) (Coward et al., 2011).

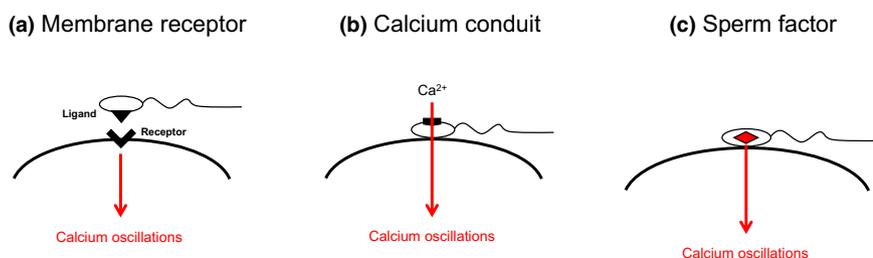


FIGURE 1 Hypotheses regarding the sperm-inducing calcium release during fertilization in mammals

TABLE 1 Mammalian species studied for induction of oocyte activation by PLC ζ or PAWP

Species	PLC ζ	PAWP ^a
Mouse	Saunders et al. (2002) Yoda et al. (2004) Fujimoto et al. (2004) Coward et al. (2006) Kurokawa et al. (2007) Ross et al. (2008) Bedford-Guaus, Yoon, Fissore, Choi, and Hinrichs (2008) Sato et al. (2013)	Wu et al. (2007)
Human	Cox et al. (2002) Yu, Saunders, Lai, and Swann (2008) Ito, Shikano, Oda, et al. (2008) Yoon et al. (2012) Sato et al. (2013)	Wu et al. (2007)
Rat	Ito, Shikano, Oda, et al. (2008) Sato et al. (2013)	
Cow	Cooney et al. (2010) Sato et al. (2013)	Wu et al. (2007)
Horse	Bedford-Guaus et al. (2011) Sato et al. (2013)	
Pig	Yoneda et al. (2006) Nakai et al. (unpubl.)	
Monkey	Cox et al. (2002)	

^aSuccessful molecular cloning was reported, but microinjection of protein or cRNA was not examined.

The injection of pufferfish PLC ζ crab into mouse oocytes failed to trigger calcium oscillations, unlike other animal species PLC ζ . Interestingly, PLC ζ is not expressed in pufferfish testes but is expressed in the ovaries, suggesting that its mechanism of action and physiological role at fertilization may differ from vertebrate species.

3 | THE STRUCTURE AND FUNCTION OF PLC ζ

There are 13 known mammalian PLC isozymes, and they are classified according to their structure into six types: PLC-beta (PLC β 1–4), -gamma (PLC γ 1, 2), -delta (PLC δ 1, 3, 4), -epsilon (PLC ϵ), -zeta (PLC ζ), and -eta (PLC η 1, 2) (Fukami, Inanobe, Kanemaru, & Nakamura, 2010; Kadampur & Ross, 2013; Suh et al., 2008), of which PLC ζ is the smallest in molecular size (Rebecchi & Pentylala, 2000). PLC ζ is characterized by its structure: it is composed of four EF-hand (EF1–EF4) domains in the N terminus, catalytic X and Y domains, and a

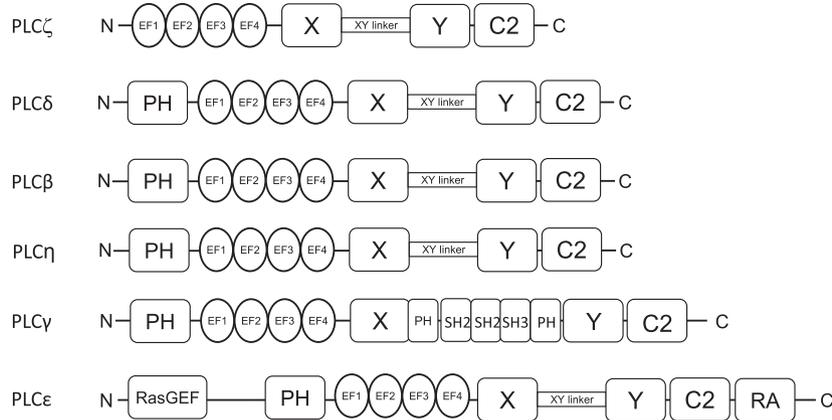
C2 domain in the C terminus that is common to other types of PLC (Figure 2a). However, PLC ζ lacks the N-terminal PH domain that is found in PLC β , - γ , - δ , and - ϵ , and the N-terminal PH domain is the site for the interaction of PLC β , - γ , - δ , and - ϵ with membrane phospholipids (Katan, 1998). PLC ζ produces IP $_3$, which binds to IP $_3$ receptor on the endoplasmic reticulum and induces the release of calcium via the hydrolysis of membrane PI(4,5)P $_2$ (Figure 3). It is thus not yet known how PLC ζ , which lacks the PH domain, binds to phosphoinositides.

PLC ζ has two functional properties: the induction of high calcium oscillations, and high calcium sensitivity in the PI(4,5)P $_2$ hydrolyzing activity. PLC ζ and PLC δ 1, which exhibits comparatively high homology with PLC ζ , have similar affinity for PI(4,5)P $_2$, but PLC ζ is extremely sensitive to calcium compared to PLC δ 1 (Nomikos et al., 2005). These properties may depend on the N-terminal EF-hand domains. The absence of all of the EF hands in PLC ζ resulted in a complete loss of both the PI(4,5)P $_2$ hydrolyzing activity and the calcium oscillation-inducing activity (Kouchi et al., 2005).

EF1 and EF2 are important for the PLC ζ activity, as the deletion of EF1 and EF2 (PLC ζ Δ EF1-2) remarkably reduced the PLC ζ activity (Kouchi et al., 2005). However, a point mutation in EF1 or EF2 did not cause a marked decline in the calcium sensitivity of the PI(4,5)P $_2$ -hydrolyzing activity (Kouchi et al., 2005). It has thus been suggested that the two EF-hand domains (rather than a calcium-binding site) play a structural role in the activation of the catalytic activity (Kouchi et al., 2005). On the other hand, the short-PLC ζ (s-PLC ζ), which lacks the three EF-hand domains EF1, EF2, and EF3, has much less calcium oscillation-inducing activity than PLC ζ Δ EF1-2 (Kouchi et al., 2005), and thus the EF3 domain is considered responsible for the high calcium sensitivity of the PI(4,5)P $_2$ -hydrolyzing activity of PLC ζ , and the EF3 domain is thought to have the important role of the induction of calcium oscillations (Kuroda et al., 2006). The enzymatic activity of PLC ζ seems to require a specific matching in the PLC ζ molecule between the four EF-hand domains and the other region, because the replacement of PLC ζ in the region from the X domain to the C terminus with PLC δ 1 resulted in no detectable PLC activity even when the four EF-hand domains were normally prepared (Kouchi et al., 2005).

The C2 domain of PLC ζ , including the 37 external amino acids in the C terminal, is a prerequisite for PLC activity and the induction of calcium oscillations. The deletion of the C2 domain (PLC ζ Δ C2) resulted in a complete loss of PLC activity (Kouchi et al., 2005). Moreover, the PLC ζ Δ C2 was unable to trigger the calcium rise (Kouchi et al., 2005; Nomikos et al., 2005). The C2 domain has been characterized as a membrane-associated and intermolecular interaction domain in a variety of proteins (Medkova & Cho, 1999). The activation mechanism of PLC ζ as well as that of PLC δ is unknown, because they lack a regulatory domain such as the G protein binding site of PLC β or the SH domain of PLC γ for phosphorylation by tyrosine kinase. The C2 domain has been suggested to take part in the calcium-dependent subcellular membrane targeting by several lipid-metabolizing enzymes (Bittova, Sumandea, & Cho, 1999; Essen, Perisic, Cheung, Katan,

(a) PLC isoforms



(b) PAWP

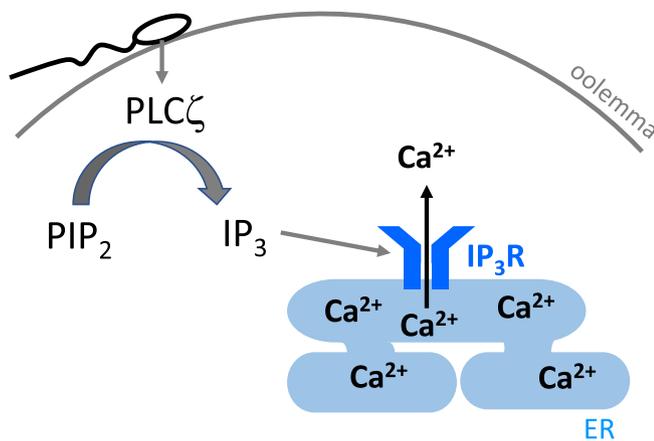


FIGURE 3 Mechanism of a rise of intracellular calcium level via PLC ζ . After the sperm-oocyte fusion, PLC ζ is released from sperm into ooplasm. The PLC ζ produces inositol trisphosphate (IP₃) by hydrolyzing phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂) and leads to release of calcium from the endoplasmic reticulum (ER) via IP₃-receptor (IP₃R)

& Williams, 1996; Grobler & Hurley, 1996; Lomasney et al., 1999; Zheng, Krishnamoorthi, Zolkiewski, & Wang, 2000). It is thus possible that the C2 domain of PLC ζ also functions as the PI(4,5)P₂ binding site. However, it has been also reported that the C2 domain does not play a direct role in the interaction with PI(4,5)P₂, because PLC ζ Δ C2 showed binding to PI(4,5)P₂ that was equivalent to that of wild-type PLC ζ (Nomikos et al., 2011), and the PLC ζ X-Y linker region, not the C2 domain, showed strong binding to PI(4,5)P₂ (Nomikos et al., 2011).

The C2 domain of PLC ζ has an affinity to PI(3)P or PI(5)P irrespective of changes in calcium (Kouchi et al., 2005; Nomikos et al., 2011). The association of the C2 domain with PI(3)P or PI(5)P may cause an inhibitory regulation of PLC activity, because the presence of PI(3)P or PI(5)P reduced the PI(4,5)P₂ hydrolytic activity of PLC ζ (Kouchi et al., 2005). The meaning of the

FIGURE 2 Structural features of PLC isoforms and PAWP. PLC ζ is composed of four EF-hand (EF1-EF4) domains in the N terminus, catalytic X and Y domains, XY linker region, and a C2 domain in the C terminus. PLC ζ is smaller than other PLC isoforms (δ , β , η , γ , ϵ). (B) The N-terminus of PAWP exhibits sequence similarity with WW domain-binding protein 2. In the proline-rich C-terminus, PAWP contains an unidentified repeating motif (YGXPPXG where X represents any residue) and a PY motifs, which is always found overlapping with the YGXPPXG motif

inhibitory effects of PI(3)P or PI(5)P on PLC ζ has not been clear, but it seems that there is a regulatory mechanism of the enzymatic activity of PLC ζ via the interaction between the C2 domain and phospholipids.

The X and Y catalytic domains are associated with the catalytic activity common to all phosphoinositide-specific PLCs. The X and Y catalytic domains were reported to be capable of binding and hydrolyzing PI(4,5)P₂, because even the X and Y catalytic domain alone, which is deleted from the C2 and EF hand domains of PLC ζ , exhibits well over half of the activity of the full-length PLC ζ (Nomikos et al., 2005). In addition, the point mutation of Asp²¹⁰ (which corresponds to a calcium-binding site responsible for the enzymatic activity of PLC δ 1) to arginine in the X catalytic domain of PLC ζ caused the loss of calcium oscillation-inducing activity (Kuroda et al., 2006). The X catalytic domain could thus affect the calcium oscillation-inducing activity.

One of the functional properties of PLC ζ is its nuclear translocation ability. The nuclear localization signal sequence, which is thought to be a binding site of the nuclear transport receptor, is at amino acids 374–381 in the X-Y linker region (Kuroda et al., 2006; Nakanishi et al., 2008). The putative nuclear localization signal (NLS) sequence Lys³⁷⁴-Ala³⁸³ fused with a fluorescent protein, accumulated into both the nucleoplasm and nucleoli in COS-7 cells (Kuroda et al., 2006). In addition to the X-Y linker region, the region including Trp¹³, Phe¹⁴, and Val¹⁸ in the N terminus of the EF1 domain is also essential for the translocation (Kuroda et al., 2006; Yoda et al., 2004). The s-PLC ζ lacking the three EF-hand domains (Δ EF1-3) is unable to assemble in the pronucleus (Yoda et al., 2004). However, PLC ζ Δ EF2-3 or Δ EF2-4 loses the translocation ability even when the EF1 domain is present (Kuroda et al., 2006). Therefore, all EF-hand domains, not only the EF1 domain, seem to play a role in the translocation of PLC ζ .

The PLC ζ or sperm factor accumulates in the PN (Kono, Carroll, Swann, & Whittingham, 1995; Larman, Saunders, Carroll, Lai, & Swann, 2004; Ogonuki et al., 2001; Sone et al., 2005; Yoda et al.,

2004), and the calcium oscillations in fertilized mouse oocytes cease at about the time of PN formation (Deguchi, Shirakawa, Oda, Mohri, & Miyazaki, 2000; Jones, Carroll, Merriman, Whittingham, & Kono, 1995). It has thus been suggested that the translocation of PLC ζ plays a key role in the cessation of calcium oscillations at the interphase of the cell cycle (Kono et al., 1995; Larman et al., 2004; Marangos, FitzHarris, & Carroll, 2003). However, it has been reported that the PLC ζ from human, rat, medaka fish (Ito, Shikano, Oda, et al., 2008), horse (Sato et al., 2013), and bovine (Cooney et al., 2010) is unable to accumulate in the PN. Calcium oscillations did not terminate at the time point of PN formation in mouse oocytes injected with pig PLC ζ cRNA (Yoneda & Watanabe, 2015). The s-PLC ζ , which loses the nuclear translocation ability and localizes in the cytoplasm after oocyte activation, had no negative effects on the pronucleus formation and subsequent development in mouse (Nakanishi et al., 2008). The physiological function of PLC ζ translocation to the PN remains uncertain.

4 | THE DIFFERENCES IN PLC ζ AMONG SPECIES

PLC ζ mRNA is expressed in spermatocytes in hamster (Young, Grasa, Coward, Davis, & Parrington, 2009) and in elongated spermatids in mouse, pig, and quail (Mizushima et al., 2009; Yoneda et al., 2006), but it seems that the spermatogenic cells do not acquire the oocyte activation ability and the calcium oscillation-inducing ability at the same time even when PLC ζ mRNA is expressed. In mouse and rat, elongated spermatids induce both oocyte activation (which involves the extrusion of the second polar body and the formation of more than one PN) and a transient rise of the intracellular calcium concentration, but not an oscillation pattern (Yazawa, Yanagida, Katayose, Hayashi, & Sato, 2000). Round spermatids are unable to induce either of these events in the above-cited species. Round spermatids in hamster, rabbit, and human can induce oocyte activation and an increase in the intracellular calcium concentration, in a transient pattern (Yazawa et al., 2000). The cells grow along with spermatogenesis, and the pattern of the increase in the intracellular calcium concentration changes from a transient pattern to an oscillation pattern. Although in hamster and rabbit the calcium oscillations are triggered by elongated spermatids, spermatozoa induce the oscillations in mouse and rat. In human, round spermatids have shown calcium oscillation-inducing ability (Yazawa et al., 2000). The timing of the functional maturation of spermatogenic cells differs among species.

PLC ζ has been detected in the postacrosomal region (Yoon & Fissore, 2007) or acrosomal and postacrosomal regions (Young et al., 2009) of mouse sperm, in the acrosomal region of rat sperm (Seita, Ito, & Kashiwazaki, 2009), in the equatorial (Yoon et al., 2008) or equatorial and postacrosomal regions (Heytens et al., 2009) of human sperm, in the equatorial region of bull sperm (Yoon & Fissore, 2007), in the acrosomal and postacrosomal regions of

hamster sperm (Young et al., 2009), in the acrosome, equatorial region, head-midpiece junction and tail of equine sperm (Bedford-Guaus et al., 2011), and in the postacrosomal region and tail of pig sperm (Nakai et al., 2011). The PLC ζ distribution patterns seem to be affected by sperm capacitation and the acrosome reaction. After capacitation or acrosome reaction in human, the proportion of sperm displaying the equatorial/acrosomal location of PLC ζ decreased, whereas sperm with the equatorial/postacrosomal pattern of PLC ζ localization increased (Grasa, Coward, Young, & Parrington, 2008). It is not clear that PLC ζ protein actually moves to the postacrosomal region, and it is not yet known whether the accessibility of the PLC ζ to an antibody changes during the capacitation and acrosome reaction.

Considering the possibility that a sperm factor is released into the oocyte cytoplasm even before the sperm's full entry, it can be speculated that a sperm factor would reside around the equatorial region, which is the site at which the sperm-oocyte fusion takes place during fertilization (Yanagimachi, 1994). However, in some species PLC ζ is localized at the acrosomal and/or tail regions. In mouse and hamster, the acrosomal/postacrosomal localization in uncapacitated sperm changes to postacrosomal localization after the capacitation and acrosome reaction (Young et al., 2009). The disappearance of acrosomal localization suggests that the acrosomal PLC ζ population might play a role in the acrosome reaction (Young et al., 2009). It would be interest to determine whether PLC ζ has functions other than the induction of oocyte activation.

5 | DISCOVERY OF PAWP

After the discovery of PLC ζ , evidence from many studies strongly indicated that PLC ζ is the functional sperm factor, at least in mammalian species. It is not yet known whether PLC ζ is the sole sperm factor and whether it is indispensable for oocyte activation during fertilization. In 2007, Dr. Richard Oko and his colleagues described a novel alkaline extractable protein of the sperm head that resides exclusively in the postacrosomal sheath region of the perinuclear theca (PT) (Wu et al., 2007). The protein shares the sequence homology with the N-terminal half of WW domain-binding protein 2 (WBP2), and the C-terminal half of the protein is unique and rich in proline. The protein was named PAWP (postacrosomal sheath WW domain-binding protein), and is also known as WBP2NL.

With the use of an antibody, PAWP was detected in the cytoplasmic lobe of spermatids beginning to undergo elongation in mouse, rabbit, pig, cow, rhesus monkey, and human (Aarabi et al., 2014a, 2014b; Wu et al., 2007). The microinjection of recombinant human PAWP or alkaline PT extract into metaphase II-arrested porcine, cow, macaque, and *Xenopus* oocytes was examined in these studies, and after microinjection, the oocytes formed pronuclei at a high rate, suggesting that PAWP could be a candidate sperm factor for oocyte activation. The co-injection of PAWP and the PAWP-derived competitive to Pro-Pro-X-Tyr (PY) motif (where X represents any residue) suppressed the induction

of pronuclear formation. Since a co-injection of PAWP with the PY motif point-mutated peptide can also induce pronuclear formation, one residue of PAWP is important for oocyte activation. The importance of one residue is same with PLC ζ because the calcium induced activity of PLC ζ was defective when Asp²¹⁰ in the X catalytic domain was mutated (Kuroda et al., 2006). Moreover, the ICSI of porcine oocytes combined with a co-injection of the competitive PY peptide or an anti-recombinant PAWP antiserum prevented pronuclear formation and arrested fertilization (Aarabi et al., 2014a, 2014b). These findings supported the hypothesis that PAWP has a crucial role as a sperm factor.

The next concern was whether PAWP can induce one or more increases in the level of calcium during oocyte activation, because such an ability is one of the most important factors for a candidate sperm factor. Aarabi, Qin, Xu, Mewburn, and Oko (2010) demonstrated that in *Xenopus* oocytes, an injection of recombinant human PAWP causes an intracellular calcium release. Their later study confirmed that an injection of human PAWP protein triggers calcium oscillations and pronuclear formation even in mammalian oocytes (mouse and human) (Aarabi et al., 2014b). In that study, the authors also showed that the sperm-induced calcium oscillations can be blocked by a co-injection of a competitive PY peptide. It suggests that both sperm-derived PAWP and an oocyte-derived group-I WW domain (WWI domain)-containing protein, such as "Yes-Associated Protein (YAP)", "neural precursor cell expressed developmentally down-regulated protein (NEDD4)", and "dystrophin", are essential for successful fertilization via calcium oscillations. These results more strongly suggested that PAWP is a sperm factor like PLC ζ .

6 | THE STRUCTURE AND FUNCTION OF PAWP

The molecular cloning of PAWP has been reported in human, mouse, and cow (Wu et al., 2007). These clones consisted of 309 (human), 354 (mouse), and 313 amino acids (cow). The N-terminal half of PAWP shares 49% sequence similarity with WBP2. Wu et al. (2007) showed that the C-terminal half of PAWP contains an unidentified repeating Tyr-Gly-X-Pro-Pro-X-Gly (YGXPPXG where X represents any residue) motif. Two (the number is species-variable) PY motifs representing a consensus binding motif for WWI domain are also present in this region of PAWP (Figure 2b).

Although it has been shown that an injection of PAWP protein or cRNA can induce calcium oscillations, the detailed mechanism of how PAWP can induce calcium oscillations during oocyte activation remains unclear. It has been hypothesized that PAWP is involved in induction of oocyte activation via interaction(s) with other protein(s), e.g., yes-associated protein (YAP) which has been reported to be involved in the interaction with PAWP (Wu et al., 2007). YAP is highly expressed in oocytes and possesses an SH3 binding motif (Chen & Sudol, 1995),

suggesting the possibility that PLC becomes noncanonically activated through its SH3 domain to cleave PI(4,5)P₂, as is the case in vertebrate fertilization.

The information about species differences in PAWP is very limited, but an injection of human PAWP can induce oocyte activation in *Xenopus* (Aarabi et al., 2010) and mouse and human (Aarabi et al., 2014b). Since there are some different nucleotides among the species, PAWP derived from different species may possess different activities, as PLC ζ does.

7 | FUTURE PROSPECTIVE SPERM FACTOR RESEARCH

After the discovery of PLC ζ , several independent research groups separately published papers that confirmed the important role of PLC ζ as a sperm factor (Table 1), but PAWP, the more recently identified candidate sperm factor, remains to be clarified. One of the major concerns is that only one group, Oko and his colleagues (Aarabi et al., 2014a, 2014b; Wu et al., 2007) confirmed the phenomenon caused by PAWP as a sperm factor. Another group attempted to reproduce the results reported by Dr. Oko and his colleagues (Aarabi et al., 2014a, 2014b; Wu et al., 2007), but failed to induce calcium oscillation with an injection of PAWP injection, at least in mouse (Nomikos, Sanders, et al., 2014) and human (Nomikos et al., 2015). In the latter study, the authors also did not observe the blockage of calcium oscillations by the injection of a competitive (PPGY) peptide for PAWP. The same research group was unable to hydrolyze PI(4,5)P₂ in vitro and activate or modulate PLC activity (Nomikos, Theodoridou, et al., 2014).

However, several studies demonstrated that PAWP expression is correlated with fertility in pig (Sutovsky, 2015), cow (Kennedy et al., 2014), and human (Aarabi et al., 2014b; Sutovsky, Aarabi, Miranda-Vizuete, & Oko, 2015), suggesting that PAWP may have other roles in the process of fertilization. Although we still do not know what causes opposite results from different research groups, further studies focusing on the underlying molecular mechanisms can be expected to clarify PAWP function.

Another concern is that only human PAWP protein or cRNA was used in the previous studies to examine the ability to induce calcium oscillations, although the successful molecular cloning of PAWP was reported, at least in mouse, human, and cow (Table 1, Wu et al., 2007). A similar mechanism of oocyte activation may well be shared among mammals. If PAWP can work as a true sperm factor in not only human but also other mammalian species, the molecules should be widely conserved among the species.

To resolve the controversy regarding PLC ζ and PAWP, mouse that were deficient in PLC ζ (Hachem et al., 2017; Nozawa, Satouh, Fujimoto, Oji, & Ikawa, 2018) or PAWP (Satouh, Nozawa, & Ikawa, 2015) were generated and their fertility was analyzed. Hachem et al. (2017) demonstrated that the PLC ζ -null sperm failed to trigger calcium oscillations, but some oocytes penetrated by PLC ζ -null sperm activated and developed into offspring. Nozawa et al.

(2018) also showed that PLC ζ -null male mouse sired healthy pups consistently, although the number of pups per copulation was significantly reduced. These results strongly suggest that oocyte activation may eventually occur via alternative pathways if PLC ζ is deficient.

In contrast, sperm from PAWP-deficient mouse induced oocyte activation with calcium oscillations and produced pups with the same efficiency as sperm from wild-type mouse (Satouh et al., 2015). It was recently suggested that there is a complementary factor of PAWP such as WBP2 (Hamilton et al., 2018). WBP2 is known as an ortholog of PAWP; both have a PY motif associated with the induction of oocyte activation. Expression of WBP2 has been confirmed in sperm and testis of mouse, but in human and cows it is only expressed in testis. Recombinant WBP2 injection into mouse oocytes induced oocyte activation with efficiency that was similar to that of recombinant PAWP (Hamilton et al., 2018). These experimental findings from experiments using knockout models raised the possibility that there is another oocyte activation pathway other than PLC ζ and PAWP.

It has been recognized that citrate synthase (CS) becomes to be another candidate as a sperm factor in nonmammalian species. Harada et al. (2007) purified from newt sperm a 45 kDa protein that induced oocyte activation accompanied by an intracellular Ca²⁺ increase when injected into unfertilized newt oocytes. The partial amino acid sequences of this 45 kDa protein were homologous to *X.laevis* CS. (Harada et al., 2007). They also showed that injection of porcine CS induced a Ca²⁺ increase in newt oocyte and caused oocyte activation (Harada et al., 2007). Interestingly, quail sperm also contain CS which is required for long-lasting, spiral-like Ca²⁺ oscillations within the activated oocyte, which is essential for cell cycle progression in early embryos (Mizushima et al., 2014). These findings suggest that CS is now a strong candidate for a sperm factor which is conserved at least in nonmammalian species. Very recently, development of CS knockout mouse has been reported (Kang et al., in abstract). The CS knockout mice are subfertility with as small litters as PLC ζ KO mouse. Although further experiments and the publication of the data are required, it seems that CS has more essential role in oocyte activation than PAWP in mammals.

Although the controversy regarding the mechanisms underlying fertilization has focused on calcium ion, it is believed that zinc signaling also has an important role in oocyte activation. Que et al. (2015) reported that the fertilization of a mature and zinc-enriched mouse oocyte triggered the ejection of zinc into the extracellular milieu in a series of coordinated events that they termed the 'zinc spark'. A zinc spark was also observed in human and cow oocytes following fertilization and oocyte activation induced by injection of PLC ζ (Duncan et al., 2016; Que et al., 2019). These reports established that the zinc spark is a highly conserved event, at least in several mammalian species.

It has also been shown that meiotic resumption can be triggered artificially with the use of TPEN, a chelator of zinc, and the meiotic resumption can be inhibited by overloading the mouse oocyte with zinc ionophores (Bernhardt, Kong, Kim, O'Halloran, & Woodruff,

2012; Suzuki, Yoshida, Suzuki, Okuda, & Perry, 2010). In mouse, TPEN treatment was shown to be sufficient to activate metaphase II-arrested oocytes injected with 'inactivated' sperm heads in the absence of intracellular calcium oscillations, resulting in live births after embryo transfer (Suzuki et al., 2010). Thus, full-term development seems not to be dependent on calcium release during metaphase II exit, and calcium oscillations do not assure an altered rate of full-term development compared to a single calcium rise.

The significance of the zinc spark is still unclear. Zinc ions must accumulate in the oocyte in order to spark at fertilization. Kim, Vogt, O'Halloran, and Woodruff (2010) showed that (a) zinc sparks could not be induced in immature oocytes that exist in the mouse ovary, and (b) the acute accumulation of zinc during meiotic maturation is important. Zinc spark profiles revealed that zygotes that developed into blastocysts released more zinc than those that failed to develop, and the rate of embryo development and the total cell number were higher than those of blastocysts (Zhang, Duncan, Que, O'Halloran, & Woodruff, 2016). It has thus been speculated that in mouse models, the amount of zinc ions at the zinc sparks can serve as an early biomarker of zygote quality. Based on the above-described findings, zinc signaling also should be part of discussions about which molecule(s) are a true sperm factor. We believe that PLC zeta plays a major role in oocyte activation as sperm factor, but oocyte activation is not necessarily due to PLC zeta alone. Future research designs should thus consider that induction of oocyte activation may be due to multiple factors and pathways.

ACKNOWLEDGMENTS

We apologize for many researchers whose work can not be cited due to space limitation. This study was partially supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS) (KAKENHI, 15H04584 to J.I., 17K08054 to N.K.). This study was supported by MEXT*-Supported Program for the Private University Research Branding Project, (2016-2019) to J.I. (*Ministry of Education, Culture, Sports, Science and Technology).

ORCID

Junya Ito  <https://orcid.org/0000-0001-9398-7358>

REFERENCES

- Aarabi, M., Balakier, H., Bashar, S., Moskovtsev, S. I., Sutovsky, P., Librach, C. L., & Oko, R. (2014a). Sperm content of postacrosomal WW binding protein is related to fertilization outcomes in patients undergoing assisted reproductive technology. *Fertility and Sterility*, 102(2), 440–447. <https://doi.org/10.1016/j.fertnstert.2014.05.003>
- Aarabi, M., Balakier, H., Bashar, S., Moskovtsev, S. I., Sutovsky, P., Librach, C. L., & Oko, R. (2014b). Sperm-derived WW domain-binding protein, PAWP, elicits calcium oscillations and oocyte activation in humans and mice. *FASEB Journal*, 28(10), 4434–4440. <https://doi.org/10.1096/fj.14-256495>
- Aarabi, M., Qin, Z., Xu, W., Mewburn, J., & Oko, R. (2010). Sperm-borne protein, PAWP, initiates zygotic development in *xenopus laevis* by eliciting intracellular calcium release. *Molecular Reproduction and Development*, 77(3), 249–256. <https://doi.org/10.1002/mrd.21140>

- Bedford-Guaus, S. J., McPartlin, L. A., Xie, J., Westmiller, S. L., Buffone, M. G., & Roberson, M. S. (2011). Molecular cloning and characterization of phospholipase C ζ in equine sperm and testis reveals species-specific differences in expression of catalytically active protein. *Biology of Reproduction*, *85*(1), 78–88. <https://doi.org/10.1095/biolreprod.110.089466>
- Bedford-Guaus, S. J., Yoon, S. J., Fissore, R. A., Choi, Y. H., & Hinrichs, K. (2008). Microinjection of mouse phospholipase C zeta complementary RNA into mare oocytes induces long-lasting intracellular calcium oscillations and embryonic development. *Reproduction, Fertility and Development*, *20*(8), 875–883. <https://doi.org/10.1071/RD08115>
- Bernhardt, M. L., Kong, B. Y., Kim, A. M., O'Halloran, T. V., & Woodruff, T. K. (2012). A zinc-dependent mechanism regulates meiotic progression in mammalian oocytes. *Biology of Reproduction*, *86*(4), 114. <https://doi.org/10.1095/biolreprod.111.097253>
- Bittova, L., Sumandea, M., & Cho, W. (1999). A structure-function study of the C2 domain of cytosolic phospholipase A2. Identification of essential calcium ligands and hydrophobic membrane binding residues. *Journal of Biological Chemistry*, *274*(14), 9665–9672. <https://doi.org/10.1074/jbc.274.14.9665>
- Carroll, D. J., Ramarao, C. S., Mehlmann, L. M., Roche, S., Terasaki, M., & Jaffe, L. A. (1997). Calcium release at fertilization in starfish oocytes is mediated by phospholipase cgamma. *Journal of Cell Biology*, *138*(6), 1303–1311. <https://doi.org/10.1083/jcb.138.6.1303>
- Chen, H. I., & Sudol, M. (1995). The WW domain of yes-associated protein binds a proline-rich ligand that differs from the consensus established for src homology 3-binding modules. *Proceedings of the National Academy of Sciences of the United States of America*, *92*(17), 7819–7823. <https://doi.org/10.1073/pnas.92.17.7819>
- Cooney, M. A., Malcuit, C., Cheon, B., Holland, M. K., Fissore, R. A., & D'Cruz, N. T. (2010). Species-specific differences in the activity and nuclear localization of murine and bovine phospholipase C ζ 1. *Biology of Reproduction*, *83*(1), 92–101. <https://doi.org/10.1095/biolreprod.109.079814>
- Coward, K., Kubota, H., Hibbitt, O., McIlhinney, J., Kohri, K., & Parrington, J. (2006). Expression of a fluorescent recombinant form of sperm protein phospholipase C zeta in mouse epididymal sperm by in vivo gene transfer into the testis. *Fertility and Sterility*, *85*(Suppl 1), 1281–1289. <https://doi.org/10.1016/j.fertnstert.2005.12.012>
- Coward, K., Ponting, C. P., Zhang, N., Young, C., Huang, C. J., Chou, C. M., ... Parrington, J. (2011). Identification and functional analysis of an ovarian form of the oocyte activation factor phospholipase C ζ (PLC ζ) in pufferfish. *Molecular Reproduction and Development*, *78*(1), 48–56. <https://doi.org/10.1002/mrd.21262>
- Cox, L. J., Larman, M. G., Saunders, C. M., Hashimoto, K., Swann, K., & Lai, F. A. (2002). Sperm phospholipase C ζ from humans and cynomolgus monkeys triggers Ca²⁺ oscillations, activation and development of mouse oocytes. *Reproduction*, *124*(5), 611–623.
- Dale, B., & Santella, L. (1985). Sperm-oocyte interaction in the sea-urchin. *Journal of Cell Science*, *74*, 153–167.
- Deguchi, R., Shirakawa, H., Oda, S., Mohri, T., & Miyazaki, S. (2000). Spatiotemporal analysis of Ca(2+) waves in relation to the sperm entry site and animal-vegetal axis during Ca(2+) oscillations in fertilized mouse oocytes. *Developmental Biology*, *218*(2), 299–313. <https://doi.org/10.1006/dbio.1999.9573>
- Ducibella, T., Schultz, R. M., & Ozil, J. P. (2006). Role of calcium signals in early development. *Seminars in Cell and Developmental Biology*, *17*(2), 324–332. <https://doi.org/10.1016/j.semcdb.2006.02.010>
- Duncan, F. E., Que, E. L., Zhang, N., Feinberg, E. C., O'Halloran, T. V., & Woodruff, T. K. (2016). The zinc spark is an inorganic signature of human oocyte activation. *Scientific Reports*, *26*(6), 24737. <https://doi.org/10.1038/srep24737>
- Essen, L. O., Perisic, O., Cheung, R., Katan, M., & Williams, R. L. (1996). Crystal structure of a mammalian phosphoinositide-specific phospholipase C delta. *Nature*, *380*(6575), 595–602. <https://doi.org/10.1038/380595a0>
- Fujimoto, S., Yoshida, N., Fukui, T., Amanai, M., Isobe, T., Itagaki, C., ... Perry, A. C. (2004). Mammalian phospholipase czeta induces oocyte activation from the sperm perinuclear matrix. *Developmental Biology*, *274*(2), 370–383. <https://doi.org/10.1016/j.ydbio.2004.07.025>
- Fukami, K., Inanobe, S., Kanemaru, K., & Nakamura, Y. (2010). Phospholipase C is a key enzyme regulating intracellular calcium and modulating the phosphoinositide balance. *Progress in Lipid Research*, *49*(4), 429–437. <https://doi.org/10.1016/j.plipres.2010.06.001>
- Grasa, P., Coward, K., Young, C., & Parrington, J. (2008). The pattern of localization of the putative oocyte activation factor, phospholipase C ζ , in uncapacitated, capacitated, and ionophore-treated human spermatozoa. *Human Reproduction*, *23*(11), 2513–2522. <https://doi.org/10.1093/humrep/den280>
- Grobler, J. A., & Hurley, J. H. (1996). Expression, characterization, and crystallization of the catalytic core of rat phosphatidylinositide-specific phospholipase C delta 1. *Protein Science*, *5*(4), 680–686. <https://doi.org/10.1002/pro.5560050412>
- Hachem, A., Godwin, J., Ruas, M., Lee, H. C., Ferrer Buitrago, M., Ardestani, G., ... Parrington, J. (2017). PLC ζ is the physiological trigger of the Ca (2+) oscillations that induce embryogenesis in mammals but conception can occur in its absence. *Development*, *144*(16), 2914–2924. <https://doi.org/10.1242/dev.150227>
- Hamilton, L. E., Suzuki, J., Acteau, G., Shi, M., Xu, W., Meinsohn, M. C., ... Oko, R. (2018). WBP2 shares a common location in mouse spermatozoa with WBP2NL/PAWP and like its descendent is a candidate mouse oocyte-activating factor. *Biology of Reproduction*, *99*(6), 1171–1183. <https://doi.org/10.1093/biolre/iy0156>
- Harada, Y., Matsumoto, T., Hirahara, S., Nakashima, A., Ueno, S., Oda, S., ... Iwao, Y. (2007). Characterization of a sperm factor for oocyte activation at fertilization of the newt *Cynops pyrrhogaster*. *Developmental Biology*, *306*(2), 797–808. <https://doi.org/10.1016/j.ydbio.2007.04.019>
- Heytens, E., Parrington, J., Coward, K., Young, C., Lambrecht, S., Yoon, S. Y., ... De Sutter, P. (2009). Reduced amounts and abnormal forms of phospholipase C ζ (PLC ζ) in spermatozoa from infertile men. *Human Reproduction*, *24*(10), 2417–2428. <https://doi.org/10.1093/humrep/dep207>
- Hinrichs, K., Choi, Y. H., Love, C. C., Chung, Y. G., & Varner, D. D. (2006). Production of horse foals via direct injection of roscovitine-treated donor cells and activation by injection of sperm extract. *Reproduction*, *131*(6), 1063–1072. <https://doi.org/10.1530/rep.1.01095>
- Ito, J., Parrington, J., & Fissore, R. A. (2011). PLC ζ and its role as a trigger of development in vertebrates. *Molecular Reproduction and Development*, *78*(10–11), 846–853. <https://doi.org/10.1002/mrd.21359>
- Ito, M., Shikano, T., Kuroda, K., & Miyazaki, S. (2008). Relationship between nuclear sequestration of PLC ζ and termination of PLC ζ -induced Ca²⁺ oscillations in mouse oocytes. *Cell Calcium*, *44*(4), 400–410. <https://doi.org/10.1016/j.ceca.2008.02.003>
- Ito, M., Shikano, T., Oda, S., Horiguchi, T., Tanimoto, S., Awaji, T., ... Miyazaki, S. (2008). Difference in Ca²⁺ oscillation-inducing activity and nuclear translocation ability of PLCZ1, an oocyte-activating sperm factor candidate, between mouse, rat, human, and medaka fish. *Biology of Reproduction*, *78*(6), 1081–1090. <https://doi.org/10.1095/biolreprod.108.067801>
- Jones, K. T. (2005). Mammalian oocyte activation: From Ca²⁺ spiking to cell cycle progression. *Reproduction*, *130*(6), 813–823.
- Jones, K. T., Carroll, J., Merriman, J. A., Whittingham, D. G., & Kono, T. (1995). Repetitive sperm-induced Ca²⁺ transients in mouse oocytes are cell cycle dependent. *Development*, *121*(10), 3259–3266.
- Kadamur, G., & Ross, E. M. (2013). Mammalian phospholipase C. *Annual Review of Physiology*, *75*, 127–154. <https://doi.org/10.1146/annurev-physiol-030212-183750>

- Katan, M. (1998). Families of phosphoinositide-specific phospholipase C: Structure and function. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, 1436(1–2), 5–17. [https://doi.org/10.1016/S0005-2760\(98\)00125-8](https://doi.org/10.1016/S0005-2760(98)00125-8)
- Kennedy, C. E., Krieger, K. B., Sutovsky, M., Xu, W., Vargovic, P., Didion, B. A., ... Sutovsky, P. (2014). Protein expression pattern of PAWP in bull spermatozoa is associated with sperm quality and fertility following artificial insemination. *Molecular Reproduction and Development*, 81(5), 436–449. <https://doi.org/10.1002/mrd.22309>
- Kim, A. M., Vogt, S., O'Halloran, T. V., & Woodruff, T. K. (2010). Zinc availability regulates exit from meiosis in maturing mammalian oocytes. *Nature Chemical Biology*, 6(9), 674–681. <https://doi.org/10.1038/nchembio.419>
- Kim, S. T., & Gye, M. C. (2003). Activation of mouse oocytes following intracytoplasmic injection of chicken sperm extract. *Reproduction in Domestic Animals*, 38(5), 401–404. <https://doi.org/10.1046/j.1439-0531.2003.00455.x>
- Kline, D., & Kline, J. T. (1992). Repetitive calcium transients and the role of calcium in exocytosis and cell cycle activation in the mouse oocyte. *Developmental Biology*, 149(1), 80–89. [https://doi.org/10.1016/0012-1606\(92\)90265-I](https://doi.org/10.1016/0012-1606(92)90265-I)
- Knott, J. G., Kurokawa, M., Fissore, R. A., Schultz, R. M., & Williams, C. J. (2005). Transgenic RNA interference reveals role for mouse sperm phospholipase c ζ in triggering Ca²⁺ oscillations during fertilization. *Biology of Reproduction*, 72(4), 992–996. <https://doi.org/10.1095/biolreprod.104.036244>
- Kono, T., Carroll, J., Swann, K., & Whittingham, D. G. (1995). Nuclei from fertilized mouse embryos have calcium-releasing activity. *Development*, 121(4), 1123–1128.
- Kouchi, Z., Fukami, K., Shikano, T., Oda, S., Nakamura, Y., Takenawa, T., & Miyazaki, S. (2004). Recombinant phospholipase c ζ has high Ca²⁺ sensitivity and induces Ca²⁺ oscillations in mouse oocytes. *Journal of Biological Chemistry*, 279(11), 10408–10412. <https://doi.org/10.1074/jbc.M313801200>
- Kouchi, Z., Shikano, T., Nakamura, Y., Shirakawa, H., Fukami, K., & Miyazaki, S. (2005). The role of EF-hand domains and C2 domain in regulation of enzymatic activity of phospholipase c ζ . *The Journal of Biological Chemistry*, 280(22), 21015–21021. <https://doi.org/10.1074/jbc.M412123200>
- Kuroda, K., Ito, M., Shikano, T., Awaji, T., Yoda, A., Takeuchi, H., ... Miyazaki, S. (2006). The role of X/Y linker region and N-terminal EF-hand domain in nuclear translocation and Ca²⁺ oscillation-inducing activities of phospholipase c ζ , a mammalian oocyte-activating factor. *The Journal of Biological Chemistry*, 281(38), 27794–27805. <https://doi.org/10.1074/jbc.M603473200>
- Kurokawa, M., Sato, K., & Fissore, R. A. (2004). Mammalian fertilization: From sperm factor to phospholipase c ζ . *Biology of the Cell*, 96(1), 37–45. <https://doi.org/10.1016/j.biolcel.2003.11.003>
- Kurokawa, M., Sato, K., Wu, H., He, C., Malcuit, C., Black, S. J., ... Fissore, R. A. (2005). Functional, biochemical, and chromatographic characterization of the complete [Ca²⁺]_i oscillation-inducing activity of porcine sperm. *Developmental Biology*, 285(2), 376–392. <https://doi.org/10.1016/j.ydbio.2005.06.029>
- Kurokawa, M., Yoon, S. Y., Alfandari, D., Fukami, K., Sato, K., & Fissore, R. A. (2007). Proteolytic processing of phospholipase czeta and [Ca²⁺]_i oscillations during mammalian fertilization. *Developmental Biology*, 312(1), 407–418. <https://doi.org/10.1016/j.ydbio.2007.09.040>
- Larman, M. G., Saunders, C. M., Carroll, J., Lai, F. A., & Swann, K. (2004). Cell cycle-dependent Ca²⁺ oscillations in mouse embryos are regulated by nuclear targeting of PLC ζ . *Journal of Cell Science*, 117(Pt 12), 2513–2521. <https://doi.org/10.1242/jcs.01109>
- Lomasney, J. W., Cheng, H. F., Roffler, S. R., & King, K. (1999). Activation of phospholipase C delta 1 through C2 domain by a Ca(2+)-enzyme-phosphatidylserine ternary complex. *The Journal of Biological Chemistry*, 274(31), 21995–22001. <https://doi.org/10.1074/jbc.274.31.21995>
- Machaty, Z., Bonk, A. J., Kuhholzer, B., & Prather, R. S. (2000). Porcine oocyte activation induced by a cytosolic sperm factor. *Molecular Reproduction and Development*, 57(3), 290–295. [https://doi.org/10.1002/1098-2795\(200011\)57:3<290::aid-mrd11>3.0.co;2-#](https://doi.org/10.1002/1098-2795(200011)57:3<290::aid-mrd11>3.0.co;2-#)
- Malcuit, C., Knott, J. G., He, C., Wainwright, T., Parys, J. B., Robl, J. M., & Fissore, R. A. (2005). Fertilization and inositol 1,4,5-trisphosphate (IP3)-induced calcium release in type-1 inositol 1,4,5-trisphosphate receptor down-regulated bovine oocytes. *Biology of Reproduction*, 73(1), 2–13. <https://doi.org/10.1095/biolreprod.104.037333>
- Marangos, P., FitzHarris, G., & Carroll, J. (2003). Ca²⁺ oscillations at fertilization in mammals are regulated by the formation of pronuclei. *Development*, 130(7), 1461–1472. <https://doi.org/10.1242/dev.00340>
- McDougall, A., Levasseur, M., O'Sullivan, A. J., & Jones, K. T. (2000). Cell cycle-dependent repetitive Ca (2+) waves induced by a cytosolic sperm extract in mature ascidian oocytes mimic those observed at fertilization. *Journal of Cell Science*, 113(Pt 19), 3453–3462.
- Medkova, M., & Cho, W. (1999). Interplay of C1 and C2 domains of protein kinase C-alpha in its membrane binding and activation. *The Journal of Biological Chemistry*, 274(28), 19852–19861. <https://doi.org/10.1074/jbc.274.28.19852>
- Miyazaki, S., Shirakawa, H., Nakada, K., & Honda, Y. (1993). Essential role of the inositol 1,4,5-trisphosphate receptor/Ca²⁺ release channel in Ca²⁺ waves and Ca²⁺ oscillations at fertilization of mammalian oocytes. *Developmental Biology*, 158(1), 62–78. <https://doi.org/10.1006/dbio.1993.1168>
- Miyazaki, S., Shirakawa, H., Nakada, K., Honda, Y., Yuzaki, M., Nakade, S., & Mikoshiba, K. (1992). Antibody to the inositol trisphosphate receptor blocks thimerosal-enhanced Ca (2+)-induced Ca²⁺ release and Ca²⁺ oscillations in hamster oocytes. *FEBS Letters*, 309(2), 180–184. [https://doi.org/10.1016/0014-5793\(92\)81090-9](https://doi.org/10.1016/0014-5793(92)81090-9)
- Miyazaki, S., Yuzaki, M., Nakada, K., Shirakawa, H., Nakanishi, S., Nakade, S., & Mikoshiba, K. (1992). Block of Ca²⁺ wave and Ca²⁺ oscillation by antibody to the inositol 1,4,5-trisphosphate receptor in fertilized hamster oocytes. *Science*, 257(5067), 251–255. <https://doi.org/10.1126/science.1321497>
- Mizushima, S., Hiyama, G., Shiba, K., Inaba, K., Dohra, H., Ono, T., ... Sasanami, T. (2014). The birth of quail chicks after intracytoplasmic sperm injection. *Development*, 141(19), 3799–3806. <https://doi.org/10.1242/dev.111765>
- Mizushima, S., Takagi, S., Ono, T., Atsumi, Y., Tsukada, A., Saito, N., & Shimada, K. (2009). Phospholipase c ζ mRNA expression and its potency during spermatogenesis for activation of quail oocyte as a sperm factor. *Molecular Reproduction and Development*, 76(12), 1200–1207. <https://doi.org/10.1002/mrd.21097>
- Nakai, M., Ito, J., Sato, K., Noguchi, J., Kaneko, H., Kashiwazaki, N., & Kikuchi, K. (2011). Pre-treatment of sperm reduces success of ICSI in the pig. *Reproduction*, 142(2), 285–293. <https://doi.org/10.1530/REP-11-0073>
- Nakanishi, T., Ishibashi, N., Kubota, H., Inoue, K., Ogonuki, N., Ogura, A., ... Baba, T. (2008). Birth of normal offspring from mouse oocytes activated by a phospholipase c ζ protein lacking three EF-hand domains. *Journal of Reproduction and Development*, 54(4), 244–249. <https://doi.org/10.1262/jrd.19168>
- Nomikos, M., Blayney, L. M., Larman, M. G., Campbell, K., Rossbach, A., Saunders, C. M., ... Lai, F. A. (2005). Role of phospholipase C- ζ domains in Ca²⁺-dependent phosphatidylinositol 4,5-bisphosphate hydrolysis and cytoplasmic Ca²⁺ oscillations. *The Journal of Biological Chemistry*, 280(35), 31011–31018. <https://doi.org/10.1074/jbc.M500629200>
- Nomikos, M., Elgmati, K., Theodoridou, M., Georgilis, A., Gonzalez-Garcia, J. R., Nounesis, G., ... Lai, F. A. (2011). Novel regulation of PLC ζ activity via its XY-linker. *Biochemical Journal*, 438(3), 427–432. <https://doi.org/10.1042/BJ20110953>

- Nomikos, M., Sanders, J. R., Kashir, J., Sanusi, R., Buntwal, L., Love, D., ... Lai, F. A. (2015). Functional disparity between human PAWP and PLC ζ in the generation of Ca²⁺ oscillations for oocyte activation. *Molecular Human Reproduction*, 21(9), 702–710. <https://doi.org/10.1093/molehr/gav034>
- Nomikos, M., Sanders, J. R., Theodoridou, M., Kashir, J., Matthews, E., Nounesis, G., ... Swann, K. (2014). Sperm-specific post-acrosomal WW-domain binding protein (PAWP) does not cause Ca²⁺ release in mouse oocytes. *Molecular Human Reproduction*, 20(10), 938–947. <https://doi.org/10.1093/molehr/gau056>
- Nomikos, M., Theodoridou, M., Elgmati, K., Parthimos, D., Calver, B. L., Buntwal, L., ... Lai, F. A. (2014). Human PLC ζ exhibits superior fertilization potency over mouse PLC ζ in triggering the Ca(2+) oscillations required for mammalian oocyte activation. *Molecular Human Reproduction*, 20(6), 489–498. <https://doi.org/10.1093/molehr/gau011>
- Nozawa, K., Satouh, Y., Fujimoto, T., Oji, A., & Ikawa, M. (2018). Sperm-borne phospholipase C ζ -1 ensures monospermic fertilization in mice. *Scientific Reports*, 8(1), 1–10. <https://doi.org/10.1038/s41598-018-19497-6>
- Nuccitelli, R., Yim, D. L., & Smart, T. (1993). The sperm-induced Ca²⁺ wave following fertilization of the xenopus oocyte requires the production of ins(1, 4, 5)P₃. *Developmental Biology*, 158(1), 200–212. <https://doi.org/10.1006/dbio.1993.1179>
- Ogonuki, N., Sankai, T., Yagami, K., Shikano, T., Oda, S., Miyazaki, S., & Ogura, A. (2001). Activity of a sperm-borne oocyte-activating factor in spermatozoa and spermatogenic cells from cynomolgus monkeys and its localization after oocyte activation. *Biology of Reproduction*, 65(2), 351–357. <https://doi.org/10.1095/biolreprod65.2.351>
- Ozil, J. P., Banrezes, B., Toth, S., Pan, H., & Schultz, R. M. (2006). Ca²⁺ oscillatory pattern in fertilized mouse oocytes affects gene expression and development to term. *Developmental Biology*, 300(2), 534–544. <https://doi.org/10.1016/j.ydbio.2006.08.041>
- Parrington, J., Swann, K., Shevchenko, V. I., Sesay, A. K., & Lai, F. A. (1996). Calcium oscillations in mammalian oocytes triggered by a soluble sperm protein. *Nature*, 379(6563), 364–368. <https://doi.org/10.1038/379364a0>
- Que, E. L., Bleher, R., Duncan, F. E., Kong, B. Y., Gleber, S. C., Vogt, S., ... O'Halloran, T. V. (2015). Quantitative mapping of zinc fluxes in the mammalian oocyte reveals the origin of fertilization-induced zinc sparks. *Nature Chemistry*, 7(2), 130–139. <https://doi.org/10.1038/nchem.2133>
- Que, E. L., Duncan, F. E., Lee, H. C., Hornick, J. E., Vogt, S., Fissore, R. A., ... Woodruff, T. K. (2019). Bovine oocytes release zinc in response to parthenogenetic and sperm-induced oocyte activation. *Theriogenology*, 127, 41–48. <https://doi.org/10.1016/j.theriogeno.2018.12.031>
- Rebecchi, M. J., & Pentylala, S. N. (2000). Structure, function, and control of phosphoinositide-specific phospholipase C. *Physiological Reviews*, 80(4), 1291–1335. <https://doi.org/10.1152/physrev.2000.80.4.1291>
- Ross, P. J., Beyhan, Z., Lager, A. E., Yoon, S. Y., Malcuit, C., Schellander, K., ... Cibelli, J. B. (2008). Parthenogenetic activation of bovine oocytes using bovine and murine phospholipase C zeta. *BMC Developmental Biology*, 8, 8–16. <https://doi.org/10.1186/1471-213X-8-16>
- Runft, L. L., Jaffe, L. A., & Mehlmann, L. M. (2002). Oocyte activation at fertilization: Where it all begins. *Developmental Biology*, 245(2), 237–254. <https://doi.org/10.1006/dbio.2002.0600>
- Sato, K., Wakai, T., Seita, Y., Takizawa, A., Fissore, R. A., Ito, J., & Kashiwazaki, N. (2013). Molecular characteristics of horse phospholipase C ζ (PLC ζ). *Animal Science Journal*, 84(4), 359–368. <https://doi.org/10.1111/asj.12044>
- Satouh, Y., Nozawa, K., & Ikawa, M. (2015). Sperm postacrosomal WW domain-binding protein is not required for mouse oocyte activation. *Biology of Reproduction*, 93(4), 94. <https://doi.org/10.1095/biolreprod.115.131441>
- Saunders, C. M., Larman, M. G., Parrington, J., Cox, L. J., Royle, J., Blayney, L. M., ... Lai, F. A. (2002). PLC ζ : A sperm-specific trigger of ca(2+) oscillations in oocytes and embryo development. *Development*, 129(15), 3533–3544.
- Seita, Y., Ito, J., & Kashiwazaki, N. (2009). Removal of acrosomal membrane from sperm head improves development of rat zygotes derived from intracytoplasmic sperm injection. *Journal of Reproduction and Development*, 55(5), 475–479. <https://doi.org/10.1262/jrd.20216>
- Sette, C., Bevilacqua, A., Bianchini, A., Mangia, F., Geremia, R., & Rossi, P. (1997). Parthenogenetic activation of mouse oocytes by microinjection of a truncated c-kit tyrosine kinase present in spermatozoa. *Development*, 124(11), 2267–2274.
- Sone, Y., Ito, M., Shirakawa, H., Shikano, T., Takeuchi, H., Kinoshita, K., & Miyazaki, S. (2005). Nuclear translocation of phospholipase C- ζ , an oocyte-activating factor, during early embryonic development. *Biochemical and Biophysical Research Communications*, 330(3), 690–694. <https://doi.org/10.1016/j.bbrc.2005.03.032>
- Stice, S. L., & Robl, J. M. (1990). Activation of mammalian oocytes by a factor obtained from rabbit sperm. *Molecular Reproduction and Development*, 25(3), 272–280. <https://doi.org/10.1002/mrd.1080250309>
- Stricker, S. A. (1999). Comparative biology of calcium signaling during fertilization and oocyte activation in animals. *Developmental Biology*, 211(2), 157–176. <https://doi.org/10.1006/dbio.1999.9340>
- Suh, P. G., Park, J. I., Manzoli, L., Cocco, L., Peak, J. C., Katan, M., ... Ryu, S. H. (2008). Multiple roles of phosphoinositide-specific phospholipase C isozymes. *BMB Reports*, 41(6), 415–434. <https://doi.org/10.5483/bmbrep.2008.41.6.415>
- Sutovsky, P. (2015). New approaches to boar semen evaluation, processing and improvement. *Reproduction in Domestic Animals*, 50(Suppl 2), 11–19. <https://doi.org/10.1111/rda.12554>
- Sutovsky, P., Aarabi, M., Miranda-Vizuete, A., & Oko, R. (2015). Negative biomarker based male fertility evaluation: Sperm phenotypes associated with molecular-level anomalies. *Asian Journal of Andrology*, 17(4), 554–560. <https://doi.org/10.4103/1008-682X.153847>
- Suzuki, T., Yoshida, N., Suzuki, E., Okuda, E., & Perry, A. C. (2010). Full-term mouse development by abolishing Zn²⁺-dependent metaphase II arrest without Ca²⁺ release. *Development*, 137(16), 2659–2669. <https://doi.org/10.1242/dev.049791>
- Swann, K. (1990). A cytosolic sperm factor stimulates repetitive calcium increases and mimics fertilization in hamster oocytes. *Development*, 110(4), 1295–1302.
- Swann, K. (1996). Soluble sperm factors and Ca²⁺ release in oocytes at fertilization. *Reviews of Reproduction*, 1(1), 33–39.
- Swann, K., & Parrington, J. (1999). Mechanism of Ca²⁺ release at fertilization in mammals. *Journal of Experimental Zoology*, 285(3), 267–275. [https://doi.org/10.1002/\(SICI\)1097-010X\(19991015\)285:3<267::AID-JEZ10>3.0.CO;2-P](https://doi.org/10.1002/(SICI)1097-010X(19991015)285:3<267::AID-JEZ10>3.0.CO;2-P)
- Swann, K., Saunders, C. M., Rogers, N. T., & Lai, F. A. (2006). PLC ζ (ζ): A sperm protein that triggers Ca²⁺ oscillations and oocyte activation in mammals. *Seminars in Cell and Developmental Biology*, 17(2), 264–273. <https://doi.org/10.1016/j.semcdb.2006.03.009>
- Whitaker, M. (2006). Calcium at fertilization and in early development. *Physiological Reviews*, 86(1), 25–88. <https://doi.org/10.1152/physrev.00023.2005>
- Wilding, M., Kyozuka, K., Russo, G. L., Tosti, E., & Dale, B. (1997). A soluble extract from human spermatozoa activates ascidian oocytes. *Development, Growth & Differentiation*, 39(3), 329–336. <https://doi.org/10.1046/j.1440-169x.1997.t01-2-00008.x>
- Wolosker, H., Kline, D., Bian, Y., Blackshaw, S., Cameron, A. M., Fralich, T. J., ... Snyder, S. H. (1998). Molecularly cloned mammalian glucosamine-6-phosphate deaminase localizes to transporting epithelium and lacks oscillin activity. *FASEB Journal*, 12(1), 91–99. <https://doi.org/10.1096/fasebj.12.1.91>

- Wu, A. T., Sutovsky, P., Manandhar, G., Xu, W., Katayama, M., Day, B. N., ... Oko, R. (2007). PAWP, a sperm-specific WW domain-binding protein, promotes meiotic resumption and pronuclear development during fertilization. *The Journal of Biological Chemistry*, 282(16), 12164–12175. <https://doi.org/10.1074/jbc.M609132200>
- Wu, H., He, C. L., & Fissore, R. A. (1997). Injection of a porcine sperm factor triggers calcium oscillations in mouse oocytes and bovine oocytes. *Molecular Reproduction and Development*, 46(2), 176–189. [https://doi.org/10.1002/\(sici\)1098-2795\(199702\)46:2<176::aid-mrd8>3.0.co;2-n](https://doi.org/10.1002/(sici)1098-2795(199702)46:2<176::aid-mrd8>3.0.co;2-n)
- Xu, Z., Kopf, G. S., & Schultz, R. M. (1994). Involvement of inositol 1,4,5-trisphosphate-mediated Ca^{2+} release in early and late events of mouse oocyte activation. *Development*, 120(7), 1851–1859.
- Yanagimachi, R. (1994). Mammalian fertilization. In E. Knobil, & J. D. Neill (Eds.), *The physiology of reproduction* (2nd ed., pp. 189–317). New York, NY: Raven.
- Yazawa, H., Yanagida, K., Katayose, H., Hayashi, S., & Sato, A. (2000). Comparison of oocyte activation and Ca^{2+} oscillation-inducing abilities of round/elongated spermatids of mouse, hamster, rat, rabbit and human assessed by mouse oocyte activation assay. *Human Reproduction*, 15(12), 2582–2590. <https://doi.org/10.1093/humrep/15.12.2582>
- Yoda, A., Oda, S., Shikano, T., Kouchi, Z., Awaji, T., Shirakawa, H., ... Miyazaki, S. (2004). Ca^{2+} oscillation-inducing phospholipase C ζ expressed in mouse oocytes is accumulated to the pronucleus during oocyte activation. *Developmental Biology*, 268(2), 245–257. <https://doi.org/10.1016/j.ydbio.2003.12.028>
- Yoneda, A., Kashima, M., Yoshida, S., Terada, K., Nakagawa, S., Sakamoto, A., ... Watanabe, T. (2006). Molecular cloning, testicular postnatal expression, and oocyte-activating potential of porcine phospholipase c ζ . *Reproduction*, 132(3), 393–401. <https://doi.org/10.1530/rep.1.01018>
- Yoneda, A., & Watanabe, T. (2015). Involvement of mouse and porcine PLC ζ -induced calcium oscillations in preimplantation development of mouse embryos. *Biochemical and Biophysical Research Communications*, 460(2), 476–481. <https://doi.org/10.1016/j.bbrc.2015.03.058>
- Yoon, S. Y., Eum, J. H., Lee, J. E., Lee, H. C., Kim, Y. S., Han, J. E., ... Yoon, T. K. (2012). Recombinant human phospholipase C zeta 1 induces intracellular calcium oscillations and oocyte activation in mouse and human oocytes. *Human Reproduction*, 27(6), 1768–1780. <https://doi.org/10.1093/humrep/des092>
- Yoon, S. Y., & Fissore, R. A. (2007). Release of phospholipase C ζ and $[Ca^{2+}]_i$ oscillation-inducing activity during mammalian fertilization. *Reproduction*, 134(5), 695–704. <https://doi.org/10.1530/REP-07-0259>
- Yoon, S. Y., Jellerette, T., Salicioni, A. M., Lee, H. C., Yoo, M. S., Coward, K., ... Fissore, R. A. (2008). Human sperm devoid of PLC, ζ 1 fail to induce Ca^{2+} release and are unable to initiate the first step of embryo development. *Journal of Clinical Investigation*, 118(11), 3671–3681. <https://doi.org/10.1172/JCI36942>
- Young, C., Grasa, P., Coward, K., Davis, L. C., & Parrington, J. (2009). Phospholipase C ζ undergoes dynamic changes in its pattern of localization in sperm during capacitation and the acrosome reaction. *Fertility and Sterility*, 91(5 Suppl), 2230–2242. <https://doi.org/10.1016/j.fertnstert.2008.05.021>
- Yu, Y., Saunders, C. M., Lai, F. A., & Swann, K. (2008). Preimplantation development of mouse oocytes activated by different levels of human phospholipase C zeta. *Human Reproduction*, 23(2), 365–373. <https://doi.org/10.1093/humrep/dem350>
- Zhang, N., Duncan, F. E., Que, E. L., O'Halloran, T. V., & Woodruff, T. K. (2016). The fertilization-induced zinc spark is a novel biomarker of mouse embryo quality and early development. *Scientific Reports*, 6, 22772. <https://doi.org/10.1038/srep22772>
- Zheng, L., Krishnamoorthi, R., Zolkiewski, M., & Wang, X. (2000). Distinct Ca^{2+} binding properties of novel C2 domains of plant phospholipase dalpha and beta. *The Journal of Biological Chemistry*, 275(26), 19700–19706. <https://doi.org/10.1074/jbc.M001945200>

How to cite this article: Nakai M, Ito J, Suyama A, Kageyama A, Tobar Y, Kashiwazaki N. Phospholipase C ζ (PLC ζ) versus postacrosomal sheath WW domain-binding protein (PAWP): Which molecule will survive as a sperm factor?. *Anim Sci J*. 2020;91:e13345. <https://doi.org/10.1111/asj.13345>